Split construct PCR

Objective : Obtain the split Luc fragments and split GFP fragments as well as the whole proteins with the linker attached on the N-terminal

Protocol : PCR protocol for Phusion High Fidelity Polymerase(50ul reactions)

First Try, 22/07/14 : No modifications – 3 out of 6 successful(Luc, NGFP and CGFP)

	Nluc	Cluc	Luc	NGFP	CGFP	GFP	+	-
Master Mix	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Fwd	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Template	0.17	0.17	0.21	0.5	0.5	0.5	0.2	0
Water	33.33	33.33	33.29	33	33	33	33.3	33.5

(Master Mix was made with 85ul 5X Phusion buffer, 8.5ul dNTP mix and 4.25ul Phusion High Fidelity Polymerase)

Result :

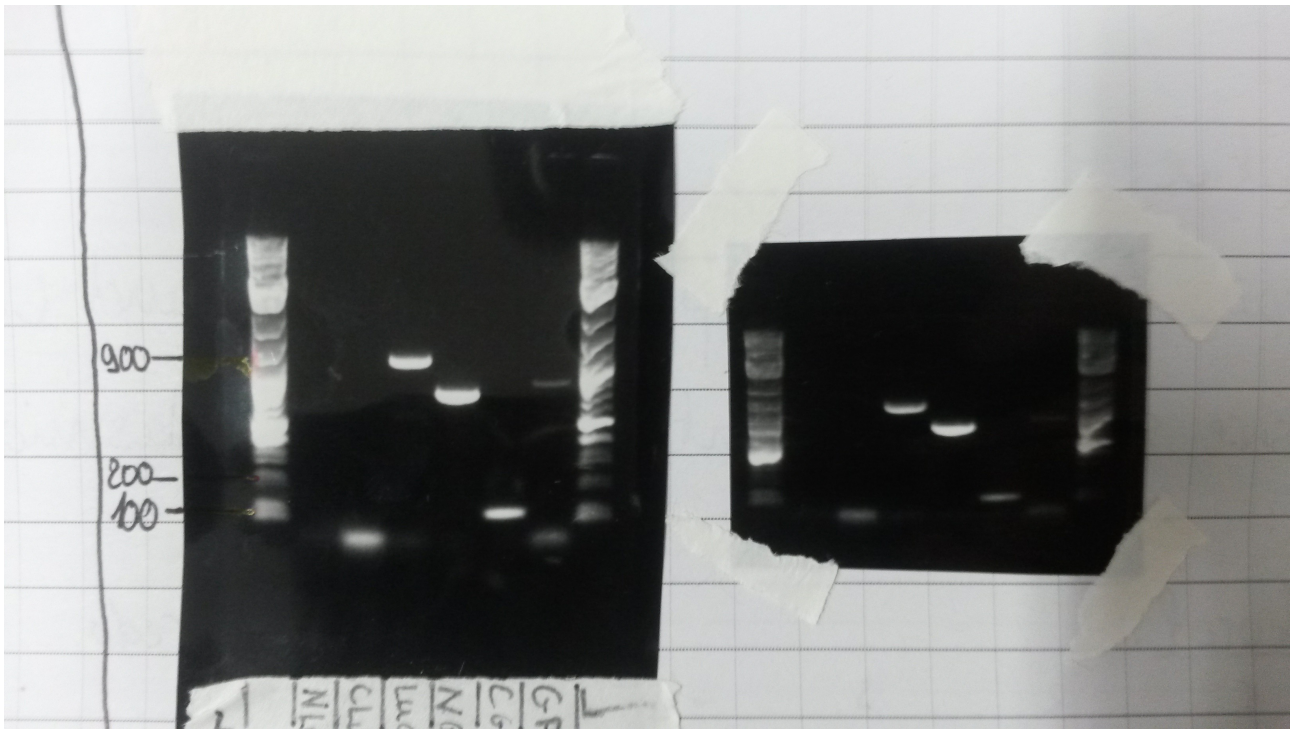


Figure 1) Agarose gel(using GelRed) of PCR products(Gel loading conditions at end of document)

Expected sizes : Nluc – 327bp + linker, Cluc – 606bp + linker, Luc – 933bp+linker, NGFP – 720bp + linker, CGFP – 75bp + linker and GFP -795bp +linker

Fragments at right height : Luc, NGFP, cGFP

Programs :

Second Try, 23/07/14 : Doubling primer concentrations

	Nluc1	Nluc2	Cluc1	Cluc2	Nluc3	Cluc3	GFP
Master Mix	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Fwd	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Template	0.17	0.17	0.17	0.17	0.17	0.17	0.5
Water	33.33	33.33	33.29	33	33	33	33.3
DMSO	0	0	0	0	1.5	1.5	0

(Primer concentrations were 1um instead of 0.5um)

Result : No band at all was visible

Third Try, 24/07/14 : Doubling primer concentrations for plasmid1, small modification to template quantity

	Nluc Plasmid1	Nluc Plasmid2	Cluc Plasmid1	Cluc Plasmid2	Nluc Amp	Cluc Amp	GFP1	GFP2
Master Mix	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Fwd	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Template	0.4	0.2	0.4	0.2	0.2	0.2	0.5	0.5
Water	33.1	33.3	33.1	33.3	33.3	33.3	33.3	33.3
DMSO	0	0	0	0	0	0	0	0

(Plasmid indicates that the template comes from the mini-prepped plasmid, Amp is the amplicon from another group)

Results :
(Expected fragment sizes can be found in first try)
A fragment at the right height was found only for the Cluc Plasmid2.

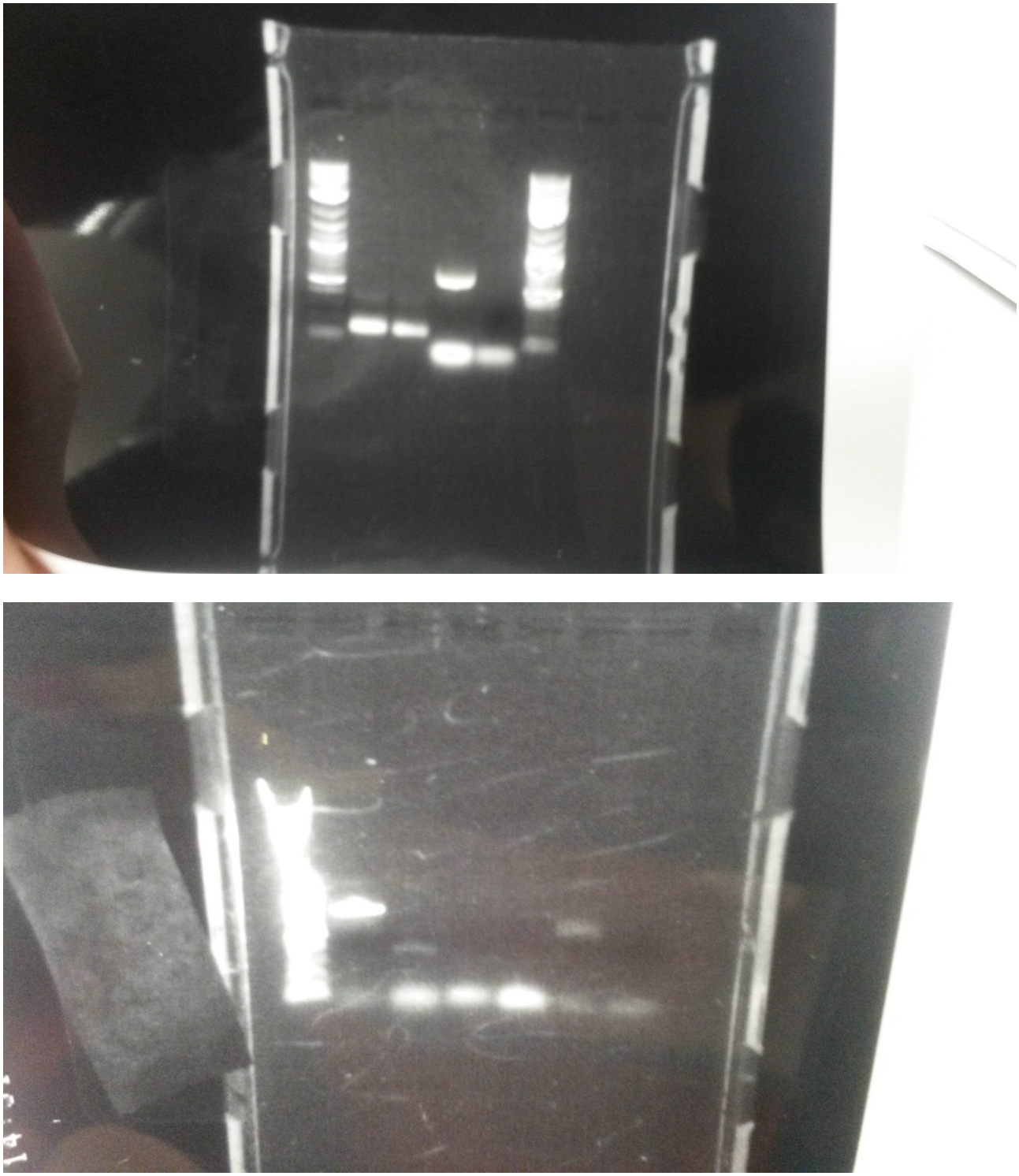


Figure 2) Agarose gel(using GelRed) of PCR products(Gel loading conditions at end of document) for the third try

Fourth Try : Template quantity variation, primer concentration back to normal

	Nluc 1	Nluc 2	Nluc 3	Nluc 4	GFP1	GFP2
Master Mix	11.5	11.5	11.5	11.5	11.5	11.5
Fwd	2.5	2.5	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5	2.5	2.5
Template	0.2	0.4	0.2	0.4	0.25	0.5
Water	33.3	33.1	31.8	31.6	33.25	33
DMSO	0	0	1.5	1.5	0	0

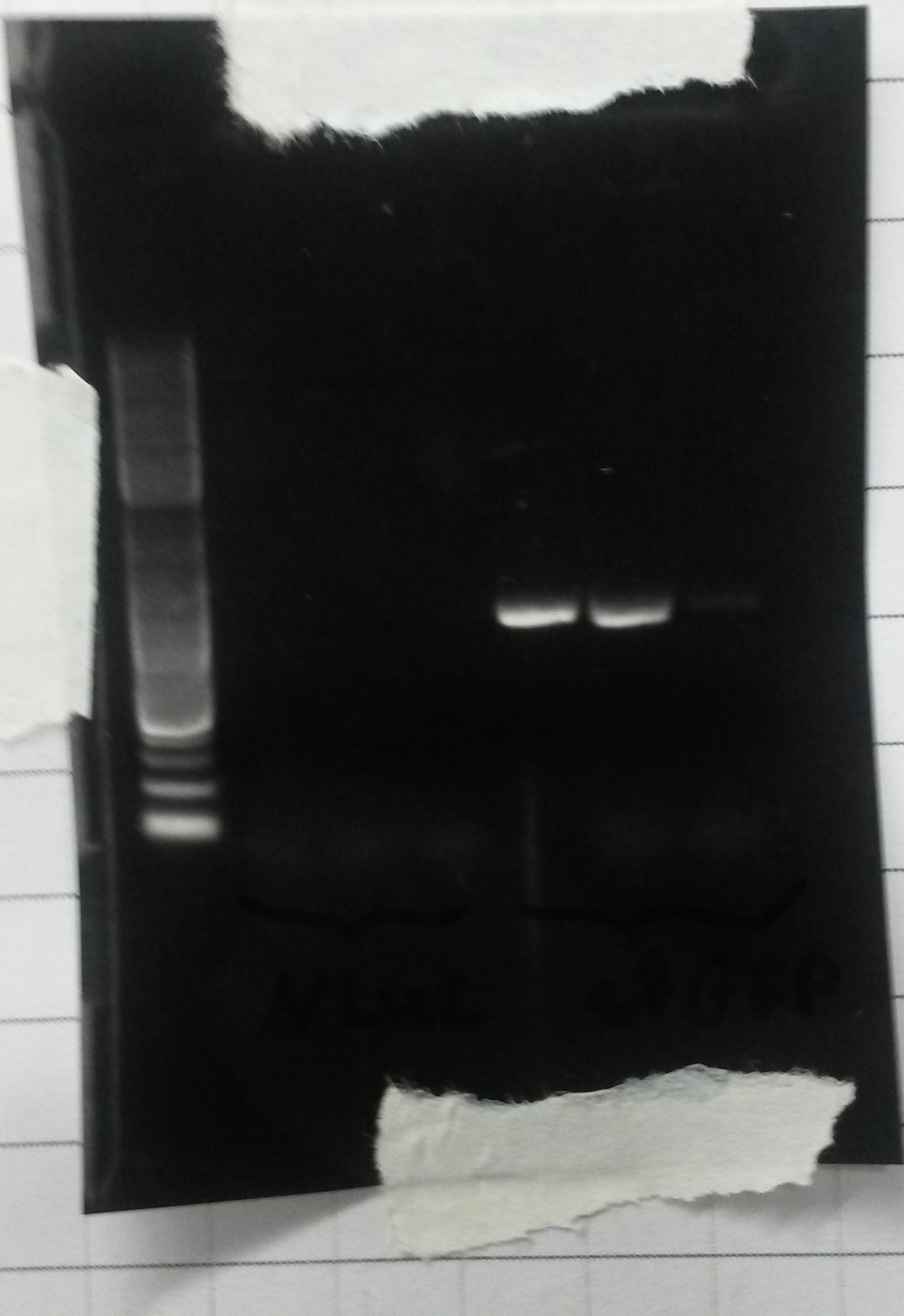
Results : No bands at all on the gel

Fifth Try : GC buffer, plasmids were replaced with newly mini-prepped ones – GFP pcr worked

	Nluc 1	Nluc 2	Nluc 3	GFP1	GFP2	GFP3
Master Mix	11.5	11.5	11.5	11.5	11.5	11.5
Fwd	2.5	2.5	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5	2.5	2.5
Template	0.2	0.2	0.2	0.2	0.2	0.2
Water	33.3	33.3	33.3	33.3	33.3	33
DMSO	0	0	0	0	0	0

Results : GFP gave bands on all three

Nluc



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th try

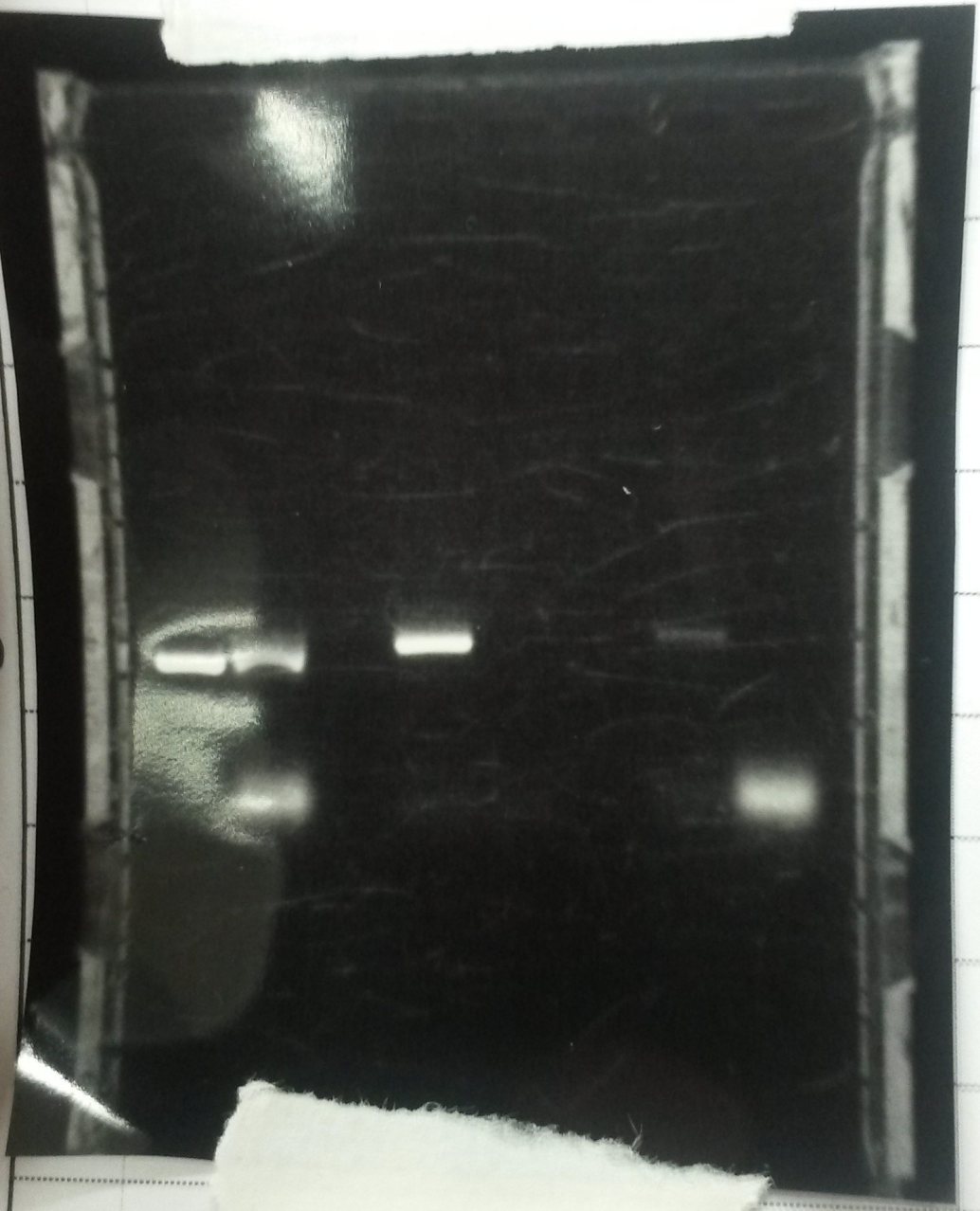
Sixth Try : Retry with GC buffer on Nluc

	Nluc1	Nluc2	Nluc3	Nluc4
Master Mix	11.5	11.5	11.5	11.5
Fwd	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5
Template	0.2	0.2	0.2	0.2
Water	33.3	31.8	33.2	33.1
DMSO	0	1.5	0	0

Seventh try : Primer test + temp change test

	Nluc 1 (+Control) CheY primers	Nluc 2 (-Control) Our primers	Nluc 3 Our fw CheY rv	Nluc4 Our rv CheY fw	Nluc5 Our primers 60°	Nluc6 Our primers 57°
Master Mix	11.5 Phusion	11.5 GC	11.5 GC	11.5 Phusion	11.5 GC	11.5 GC
Fwd	2.5	2.5	2.5	2.5	2.5	2.5
Rev	2.5	2.5	2.5	2.5	2.5	2.5
Template	0.2	0.2	0.2	0.2	0.2	0.2
Water	33.3	33.3	33.3	33.3	33.3	33
DMSO	0	0	0	0	0	0

Results : Fragments for lanes 1 and 3



SIGNATURE

Concludes that our reverse primer was faulty